REMARKS

Claims 1, 6-47, 49-53, and 56 are pending. Claims 10, 13-47, and 49-53 are withdrawn. Claims 2-5, 48, 54 and 55 have been canceled. Claim 56 has been added. Claim 1 was amended to indicate that the method is directed to determining the toxicity of a test compound by monitoring the effect of the test compound on cell differentiation of pluripotent stem cells, that the undifferentiated pluripotent stem cells are contacted by the test compound; that the pluripotent stem cells are differentiated into at least one type of differentiated cells; that the amount or activity of the secreted reporter gene product within the cell culture medium is correlated with the proportion of differentiated cells to undifferentiated pluripotent stem cells; and that the toxicity of the test compound is determined by comparing the proportion of the differentiated cells in the cell culture medium in the presence of the test compound with a control cell culture medium lacking the test compound, wherein a decreased proportion of differentiated cells in the presence of the test compound relative to the control is indicative of the test compound's toxicity. Support for this amendment is found in the specification, *e.g.*, on page 30, lines 8-30. Claim 7 has been amended to clarify that the cells are "differentiated" cell types. Claim 8 has been amended to be consistent with claim 6 from which it depends. Claim 56 has been added and indicates that the differentiated cell type is a cardiomyocyte. Support for this is claim is found, *e.g.*, on page 13, lines 4-6 and in Example 3. No new matter has been added by these amendments. Entry of this amendment is respectfully requested.

Applicants acknowledge with appreciation the withdrawal of the rejections of claims 1, 4, 6-9 and 11-12 under 35 U.S.C. § 112, second paragraph and enablement under 35 U.S.C. §112.

Reconsideration of the remaining rejections is respectfully requested.

1. Claim Rejections – 35 U.S.C. § 112, Second Paragraph

The Office Action rejects claims 1, 4, 6-9, 11, 12 and 55 as allegedly being indefinite under 35 U.S.C. § 112, second paragraph. Claim 1 is rejected as being indefinite because allegedly there is no conclusion which is commensurate with the preamble. Office Action at page 3, third paragraph. Claims 4, 6-9, 11, 12 and 55 are rejected for depending from a rejected base claim.

Applicants have amended claim 1 to encompass "a method for determining the toxicity of a test compound by monitoring the effect of the test compound on cell differentiation of pluripotent stem cells…wherein a decreased proportion of differentiated cells in the presence of the test compound relative to the control is indicative of the test compound toxicity." Applicants believe that these amendments obviate the instant rejection and request withdrawal thereof.

2. Claim Rejections – 35 U.S.C. § 112, First Paragraph

The Office Action rejects claims 1, 4, 6-9, 11-12, and 55 under 35 U.S.C. §112, first paragraph, as allegedly failing to comply with the written description requirement. Office Action at page 3, penultimate paragraph. The claims allegedly lack written description because the claims broadly require a generic cell differentiation into at least a generic final cell type. According to the Office, given the broad listings of cells types, which indicate any differentiation of any cell of any generic cell, and the lack of specific linking between the generic cell capable of differentiating, and what they can differentiate into, there is a lack of description of the generic "conditions allowing for differentiation of said cells" for the breadth of cells encompassed prior to and upon differentiation. Office Action at page 4, first paragraph. Applicants respectfully disagree and traverse.

With regard to written description, "[t]he specification need not disclose what is well-known to those skilled in the art and preferably omits that which is well-known to those skilled and already available to the public. *In re Buchner,* 929 F.2d 660, 661, 18 USPQ2d 1331, 1332 (Fed. Cir. 1991); *Hybritech, Inc.* v. *Monoclonal Antibodies, Inc.,* 802 F.2d 1367, 1384,231 USPQ 81, 94 (Fed. Cir. 1986), *cert. denied,* 480 U.S. 947 (1987); and *Lindemann Maschinenfabrik GMBH v. American Hoist & Derrick Co.,* 730 F .2d 1452, 1463, 221 USPQ 481,489 (Fed. Cir. 1984). *See,* MPEP § 2164.05(a) (Emphasis added). The Federal Circuit also noted that the written description requirement does not require that "every invention must be described in the same way. As each field evolves, the balance also evolves between what is known and what is added by each inventive contribution." Slip op. at 17 (citing *Capon* v. *Eshhar,* 419 F.3d 1349, 1358 (Fed. Cir. 2005)). The court acknowledged that the scope of the written description for each application will vary because of the differences in the state of the knowledge in the field and in the predictability of the science. *Id.*

An adequate written description of the invention may be shown by any description of sufficient, relevant identifying characteristics so long as a person skilled in the art would recognize that the inventor had possession of the claimed invention. *See, e.g., Purdue Pharma L.P. v. Faulding Inc.,* 230 F.3d 1320, 1323, (Fed Cir. 2000). Moreover, the written description requirement does not require that the specification disclose *in haec verba* (verbatim) a phrase present in a claim but merely requires support in the specification through express, implicit, or inherent disclosure. *See* M.P.E.P. 2163(I)(B), Guidelines for the Examination of Patent Applications Under the 35 U.S.C. 112, para. 1, "Written Description" Requirement.

Claim 1, as amended herein, is directed to a method for determining the toxicity of a test compound by monitoring the effect of the text compound on cell differentiation of *pluripotent*

stem cells. The method includes: (1) culturing undifferentiated pluripotent stem cells having at least one recombinant nucleic acid molecule comprising a reporter gene encoding a product that is secreted upon cell differentiation and at least one cell type-specific promoter sequence operably linked to the reporter gene; (2) contacting the undifferentiated pluripotent stem cells with the test compound; (3) differentiating a portion of the pluripotent stem cells into at least one type of differentiated cells; (4) determining the amount or activity of the reporter gene product within the cell culture medium of said differentiated cells, wherein said reporter gene product comprises a secretory leader sequence, and wherein said secreted reporter gene product is not recaptured from said cell culture medium; (5) correlating the amount or activity of the secreted reporter gene product within the cell culture medium with a proportion of differentiated cells to undifferentiated pluripotent stem cells; and (6) determining the toxicity of the text compound by comparing the proportion of differentiated cells in the cell culture medium lacking the test compound, wherein a decreased proportion of differentiated cells in the presence of the test compound relative to the control is indicative of the test compound toxicity.

Claim 1 was further amended to delete the step directed to "determining the amount or activity of the reporter gene product within a body fluid of said transgenic non-human animal." Thus, the portion of the Office's written description rejection related to the assertion that "the specification fails to sufficiently describe the use of non-human animals" in the methods of the present claims has been rendered moot and withdrawal of the rejection is respectfully requested.

The claims, as presented herein, have been amended to methods for determining the effect of a test compound on the ability of *pluripotent stem cells* to undergo differentiation by measuring the activity or amount of a secreted reporter gene product, correlating the amount of

reporter gene product with the proportion of differentiated cells and comparing the amount of differentiated cells in the presence of the test compound to the amount of differentiated cells in a control sample. Consequently, the specification provides sufficient written description such that the skilled artisan would envision that Applicants possessed methods for determining the toxicity of a test compound on the differentiation of pluripotent stem cells within the scope of the present claims.

The specification in Example III describes a method for determining the toxicity of retinoic acid on the differentiation of embryonic stem (ES) cells into cardiac cells. According to Example III, ES cell samples were transfected with the α-MHC-SEAP vector (containing the SEAP gene under the control of the heart-specific α-MHC promoter), treated with retinoic acid at different concentrations and subjected to a differentiation protocol resulting in the generation of cardiac cells. *See* Specification at page 44, lines 7-9. Samples were then assayed to determine SEAP activity. As shown in Figure 1, a concentration-dependent effect of retinoic acid on SEAP activity in cell sample supernatants was observed. Consequently, these results indicate the suitability of the claimed methods utilizing a reporter gene product to monitor the effect of a text compound on the differentiation of ES cells.

Therefore, coupled with what was well-known in the art at the time of the earliest filing date of the instant application, the specification conveys with reasonable clarity that Applicants were in possession of a method for determining the toxicity of a test compound by monitoring the effect of the test compound on cell differentiation of pluripotent stem cells. Consequently, Applicants submit that the pending claims fully meet the written description requirements of 35 U.S.C. § 112, first paragraph.

Thus, Applicants respectfully request withdrawal of the rejection of the claims under 35 U.S.C. §112, first paragraph, as failing to comply with the written description requirement

3. Claim Rejections – 35 U.S.C. § 102

The Office has maintained the rejection of claims 1, 4, 6-9 and 11-12 under 35 U.S.C. § 102(b) as allegedly being anticipated by Benkel *et al.* (WO 98/49320) ("Benkel"). The rejection states that Benkel "teaches the advantages of using a reporter gene system for studying the regulation of gene expression that is of fundamental importance among others to cell division and cell differentiation." Applicants respectfully disagree with the characterization of the Benkel reference.

To anticipate claimed subject matter, a reference must disclose *each and every element* of the claimed invention, whether it does so explicitly or inherently. *Eli Lilly & Co. v. Zenith Goldline Pharms., Inc.*, 471 F.3d 1369, 1375 (Fed. Cir. 2006). The elements must be "arranged or combined in the same way as in the claim," *Net MoneyIN, Inc. v. VeriSign, Inc.*, 545 F.3d 1359, 1370 (Fed. Cir. 2008). The reference must also "enable one of ordinary skill in the art to make the invention without undue experimentation." *Impax Labs., Inc. v. Aventis Pharms. Inc.*, 545 F.3d 1312, 1314 (Fed. Cir. 2008); see *In re LeGrice*, 301 F.2d 929, 940–944 (C.C.P.A. 1962).

The claims, as presented herein, have been amended to methods for determining the effect of a test compound on the ability of *pluripotent stem cells* to undergo differentiation by measuring the activity or amount of a secreted reporter gene product, correlating the amount of reporter gene product with the proportion of differentiated cells and comparing the amount of

differentiated cells in the presence of the test compound to the amount of differentiated cells in a control sample.

Applicants respectfully note that the Benkel application describes a "secreted reporter gene system based on one or more alpha-amylases, which are electrophoretically distinct variants of each other, for simultaneous expression in mammalian cells." Benkel at page 1, lines 3-6. Benkel further describes that the alpha-amylase reporter gene encodes a biological activity that is easily measured by a variety of liquid or semi-solid phase assay systems. *Id.* at page 6, lines 1-2.

However, Benkel does *not* describe a method for determining the effect of a test compound on the ability of *pluripotent stem cells* to undergo differentiation by measuring the activity or amount of a secreted reporter gene product, correlating the amount of reporter gene product with the proportion of differentiated cells and comparing the amount of differentiated cells in the presence of the test compound to the amount of differentiated cells in a control sample. At most, Benkel has a *single* generic statement about cell differentiation: "The regulation of gene expression is of fundamental importance to all biological functions including adaptation to environmental conditions, cell division and differentiation, and the development of disease states such as cancer." *Id.* at Abstract.

Benkel fails to teach – let alone enable the skilled artisan – how its method could be applied to determine the effect of a test compound on the ability of *pluripotent stem cells* to undergo differentiation by measuring the activity or amount of a secreted reporter gene product. Instead, Benkel focus is on distinguishing between *transformed mammalian cells versus untransformed mammalian* cells through the electrophoretic detection of secreted α-amylases. In fact, each of the cell lines transformed in the Benkel application is *already differentiated.*

The present invention, on the other hand, is directed to determining the effect of a test compound on the differentiation of *pluripotent stem cells* by measuring the amount or activity of a secreted gene product. Nothing in Benkel teaches, suggests or enables this method as claimed.

Therefore, Benkel does not anticipate claims 1, 4, 6-9 and 11-12 because, *inter alia*, it does not teach and enable a method of monitoring the effect of a test compound on cell differentiation utilizing cells capable of differentiating. *Eli Lilly & Co. v. Zenith Goldline Pharms., Inc.*, 471 F.3d 1369, 1375; *Impax Labs., Inc. v. Aventis Pharms. Inc.*, 545 F.3d 1312, 1314. Accordingly, the anticipation rejection is improper and withdrawal thereof is respectfully requested.

4. Claim Rejections – 35 U.S.C. § 103

The Office has maintained the rejection of claims 1, 4, 6-9 and 11-12 under 35 U.S.C. §103(a) as, allegedly, being unpatentable over Benkel in view of Goldspink *et al.* (US 2003/0008836; "Goldspink") and Bronstein *et al.* (1994, *Biotechniques* 17:172-177; "Bronstein").

The Office alleges that "it would have been obvious for one of ordinary skill in the art to incorporate SEAP reporter gene of Benkel for *lacZ* gene in the reporter construct of Goldspink and follow the differentiation of stem cells to specific tissue types or cell types using [very] sensitive SEAP assays taught by Bornstein." Office Action at page 9, first paragraph. The Office also alleges that one of ordinary skill would be motivated to use an "assayable secreted reporter that will not be captured by tissues or the cells for monitoring a gene regulation during differentiation of a cell into tissue cell type as it is less invasive and avoids lysis of the cells." *Id.* Finally, the Office concludes that there would have been a "reasonable expectation of success

making [and] using recombinant progenitor or stem cell having a reporter gene construct that codes for a secretable reporter protein for evaluating and identifying the differentiated cells as the art teaches that it is routine to use a recombinant secretable reporter for marking differentiation." *Id.* Applicants respectfully disagree and traverse.

Benkel is asserted to teach the "advantages of using a reporter gene system for studying the regulation of gene expression that is of fundamental importance among others to cell division and cell differentiation" and describe "reporter genes whose expression is secretable for monitoring the same." Office Action page 8, second paragraph.

As discussed herein above in response to the rejection of claims 1, 4, 6-9 and 11-12 under 35 U.S.C. §102(b), Benkel does *not* teach methods for determining the effect of a test compound on the ability of *pluripotent stem cells* to undergo differentiation by measuring the activity or amount of a secreted reporter gene product, correlating the amount of the reporter gene product in the cell culture medium with the proportion of differentiated cells and comparing the amount of differentiated cells in the presence of the test compound to the amount of differentiated cells in a control sample. Benkel is completely silent regarding the use of a reporter gene product in determining the toxicity on cell differentiation. Instead, Benkel teaches the advantages of using the alpha-amylase reporter gene in transfection experiments.

Goldspink is relied upon to teach a "method of detecting myoblast differentiation by transfecting recombinant nucleic acid molecules encoding a human alpha-gal reporter gene under the control of promoter comprising MLC1/3 enhancer to undifferentiated myoblasts wherein the reporter gene was expressed and secreted from differentiated muscle cell in vitro culture." Office Action at page 8, penultimate paragraph.

However, Goldspink does not remedy the deficiencies identified in Benkel. Goldspink describes the transfection of undifferentiated myoblasts with cationic liposomes followed by differentiation to mature myotubes. However, nothing in Goldspink teaches a method of determining the toxicity of a compound on the differentiation of pluripotent stem cells by measuring the activity or amount of a reporter gene product.

Finally, Bornstein is relied upon to teach "improvements in the detection sensitivity of SEAP reporter using chemiluminescent assays of the secreted reporter from cells in culture or tissue [*sic*]." Office Action at page 8, last paragraph. However, Bornstein does not remedy the deficiencies identified in Benkel. Bornstein teaches the use of the secreted alkaline phosphatase (SEAP) and β-glucuronidase (GUS) reporter gene products in chemiluminescent assays. *See* Bronstein Abstract. Bornstein discusses that "[r]eporter gene assays have become essential for the study of gene regulatory elements" and that "[r]eporter genes are used as indicators for the identification of sequences and factors that control gene expression at the transcriptional level." *Id.* at Introduction. However, nothing in Bornstein teaches a method of determining the toxicity of a compound on the differentiation of pluripotent stem cells by measuring the activity or amount of a reporter gene product.

None of the references either individually, or in combination, teach or suggest to one having ordinary skill in the art that the toxicity of a compound on the differentiation of pluripotent stems cells can be determined by measuring the activity or amount of a reporter gene product. Thus, as Benkel, Goldspink and Bornstein, alone or in combination, do not disclose the presently claimed invention, the Office Action has not set forth a *prima facie* case of obviousness. Consequently, Applicants respectfully request that the rejection under 35 U.S.C. § 103(a) be reconsidered and withdrawn.

5. Secondary Considerations of Nonobviousness

Applicants respectfully submit that even if a *prima facie* case of obviousness were to have been set forth in the Office Action – which it has not – evidence of a long felt but unsolved need clearly rebuts any such showing. As set forth in the M.P.E.P.:

> Office personnel should consider all rebuttal arguments and evidence presented by applicants. *See, e.g., Soni*, 54 F.3d at 750, 34 USPQ2d at 1687 (error not to consider evidence presented in the specification). *C.f., In re Alton*, 76 F.3d 1168, 37 USPQ2d 1578 (Fed. Cir. 1996) (error not to consider factual evidence submitted to counter a 35 U.S.C. 112 rejection); *In re Beattie*, 974 F.2d 1309, 1313, 24 USPQ2d 1040, 1042-43 (Fed. Cir. 1992) (Office personnel should consider declarations from those skilled in the art praising the claimed invention and opining that the art teaches away from the invention.); *Piasecki*, 745 F.2d at 1472, 223 USPQ at 788 ("[Rebuttal evidence] may relate to any of the *Graham* factors including the so-called secondary considerations.").
>
> Rebuttal evidence may include evidence of "secondary considerations," such as "commercial success, long felt but unsolved needs, [and] failure of others." *Graham v. John Deere Co.*, 383 U.S. at 17, 148 USPQ at 467. *See also, e.g., In re Piasecki*, 745 F.2d 1468, 1473, 223 USPQ 785, 788 (Fed. Cir. 1984) (commercial success). Rebuttal evidence may also include evidence that the claimed invention yields unexpectedly improved properties or properties not present in the prior art. Rebuttal evidence may consist of a showing that the claimed compound possesses unexpected properties. *Dillon*, 919 F.2d at 692-93, 16 USPQ2d at 1901.

M.P.E.P. § 2145.

Attached herewith is a Declaration (hereinafter "Ehlich Declaration") by inventor Andreas Ehlich, which demonstrates that the results for determining the toxicity of a test compound by monitoring the effect of the test compound on cell differentiation of pluripotent embryonic stem cells were surprising and unexpected. *See* Ehlich Declaration at paragraph 20.

As described in the Ehlich Declaration, prior methods for determining the toxicity of chemicals on the differentiation of embryonic stem cells were extremely laborious and cost-

intensive. *Id.* at paragraph 16. For example, the prior art embryonic stem cell test (EST) had very low throughput of test compounds and required visual inspection of at least 24 embryoid bodies (EBs) per data point. In another prior art example, a modified EST protocol that relies on the expression of the green fluorescence protein (GFP) reporter gene was developed. According to this method, expression of GFP in embryoid bodies is measured during differentiation of embryonic stems cells (ESCs).

Although the use of GFP-based assay increased throughput by at least tenfold as compared to the EST assay, the dynamic range of the GFP assay is limited and the assay has a low signal-to-background ratio. *Id.* at paragraph 18. Moreover, the GFP assay is also burdensome in requiring the determination of about 60 EBs per data point and counting of surviving EBs before measurement. *Id.*

As described in the Ehlich Declaration, applicants surprisingly and unexpectedly discovered the claimed methods which overcome the limitations of the EST and GFP assays. By utilizing a secreted reporter gene product (e.g., secreted alkaline phosphatase (SEAP)), it was determined that, in comparison to the 24 and 60 EBs required for the EST and GFP based assays, respectively, a *single* EB can be utilized to determine the effect of a test compound on the differentiation of ESCs. *Id.* at paragraph 19. Consequently, assay throughput is significantly increased as an individual EB can be deposited into a well of a 96-well-plate compared to, for example, the use of a 6-well-plate for the GFP assay. Additionally, the signal-to-background ratio of differentiated to undifferentiated ESCs in the presently claimed methods is increased by more than one hundred fold compared to the EST assay. *Id.* at paragraph 20.

Thus, even if the Office Action had set forth a *prima facie* case of obviousness, Applicants submit that the Ehlich Declaration to support the proposition that the efficient, high-

throughput embryotoxicity assay presently claimed provides surprising and unexpectedly superior results over what could have been expected in the prior art.

Conclusion

In view of the foregoing amendments and remarks, Applicant respectfully requests reconsideration and reexamination of this application and the timely allowance of the pending claims. The Examiner is invited to telephone the undersigned if that would be helpful to resolving any issues.

It is believed that no fees are due; however, the commissioner is authorized to charge any fees and credit any overpayments to Deposit Account No. 50-5071. Additionally, please grant any extensions of time required to enter this response and charge any additional required fees to our deposit account 50-5071.

Respectfully submitted,

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